1 2

APPLIED MICROBIOLOGY, Nov., 1966 Vol. 14, No. 6

β-Propiolactone Vapor Decontamination

ROBERT K. HOFFMAN, LEE M. BUCHANAN, AND DAVID R. SPINER
U.S. Army Biological Center, Fort Detrick, Frederick, Maryland

Received for publication 27 June 1966

ABSTRACT

HOFFMAN, ROBERT K. (Fort Detrick, Frederick, Md.), LEE M. BUCHANAN, AND DAVID R. SPINER. β-Propiolactone vapor decontamination. Appl. Microbiol. 14: 989–992. 1966.—Although β-propiolactone (BPL) is an effective vapor-phase decontaminant for enclosed areas, some problems have been encountered in its use. Adequate air circulation during BPL dissemination could eliminate most of these problems. It is recommended that, when decontaminating the ordinary building or laboratory, the amount of BPL sprayed be changed from the previously suggested 1 gal/16,000 cubic ft of space to 1 gal/25,000 cubic ft. The use of aqueous BPL solutions and thermal-type generators is not recommended.

β-Propiolactone (BPL) has been shown to be an effective vapor-phase decontaminant for the treatment of enclosed areas (1–3, 6, 10). It is not only effective against bacterial spores and vegetative cells, but against viruses and rickettsiae as well (4, 5). It has been widely used in this laboratory, and, to a lesser extent, elsewhere, since its action was first reported by Hoffman and Warshowsky in 1958.

The purpose of this report is (i) to discuss some of the physical characteristics of the chemical, (ii) to point out their relationship to the problems encountered in BPL decontamination, (iii) to indicate means of avoiding the problems, and (iv) to discuss the toxicity and means of detecting trace amounts of BPL.

Because liquid BPL is a good organic solvent, problems are often encountered when it is used as a decontaminant. The principal problem is the dissolution of paints, lacquers, and waxes on surfaces in the enclosure treated. Other problems include its attack on some plastics and the occasional formation of a tacky residue, usually on horizontal surfaces. These problems are due to the liquid BPL or its aqueous solution, but not to the vapor, and thus can be eliminated by proper dissemination of the chemical. The primary considerations in BPL decontamination are discussed below.

BPL CONCENTRATION

A BPL air concentration of 1 to 1.5 mg/liter is sufficient to kill bacterial spores within minutes at 27 C (6). To obtain this amount of BPL in an enclosure, a considerably higher concentration must be disseminated (10). Originally, it was suggested that, when decontaminating an enclosure, 1 gal of BPL be dispensed for each 16,000 cubic ft of space at 25 C. Assuming no wall adsorption of the chemical and 100% efficiency of the dispenser, the theoretical air concentration would be 9.6 mg of BPL per liter (Table 1). However, the vapor pressure of BPL at 25 C is only 2.1 mm of Hg (Table 2), so the highest obtainable vapor concentration is 8.1 mg of BPL per liter of space. Thus it is evident that, at this temperature, if 1 gal of BPL is sprayed per 16,000 cubic ft of space, the amount will be above air-saturation concentration and will be six or seven times the actual concentration required to kill spores.

If excess BPL is sprayed into an area or if the dispenser emits large droplets, the lactone can literally "rain out" on surfaces, leaving a liquid coating. Since the surrounding air is close to saturation, the evaporation will at best be extremely low and the liquid will have more time to react with and to dissolve surfaces such as paints and plastics, or to polymerize, leaving a tacky residue. In an unsaturated atmosphere, the rate of evaporation from a surface is about 1.6%/min at 25 C, or a half-life of 43 min. It should be emphasized that it is only the liquid BPL that deleteriously affects paints and plastics, or leaves a tacky residue. The vapor has not been shown to be deleterious to normally encountered materials when used at recommended concentrations and exposure times.

When partially polymerized BPL (less than about 93% purity) is sprayed, a noticeable residue is occasionally produced that is difficult to remove from surfaces. This problem is not encountered with BPL of higher purity. If BPL is to be kept for

TABLE 1. *Calculated concentration in air after spraying 1 gal of BPL into various volumes*[a]

Vol	BPL concn
ft^3	*mg/liter*
12,000	12.78
16,000	9.6
20,000	7.67
25,000	6.38
30,000	5.11

[a] Assuming complete evaporation.

TABLE 2. *Vapor pressure and maximal concentration of BPL in air at various temperatures*

Temp	Vapor pressure	Concn in air[a]
C	*mm of Hg*	*mg/liter*
−10	0.12	0.5
−5	0.22	0.9
0	0.34	1.4
5	0.50	2.0
10	0.74	3.0
15	1.05	4.2
20	1.49	5.9
25	2.10	8.1
30	2.90	11.1
35	4.05	15.2

[a] Calculated from formula $PV = nRT$.

a time before use, it should be stored in the refrigerator to retard the formation of polymers.

Recent tests in this laboratory demonstrated that spraying 1 gal/25,000 cubic ft of space provides adequate decontamination. This yields a theoretical air concentration of 6.4 mg/liter of air, several milligrams below saturation level at 25 C, yet four to five times more BPL than that required for sterilization. There are several reasons why it is necessary to disseminate more BPL than that required to decontaminate, i.e., adsorption on walls, floors, and other surfaces; sprayer inefficiency; inadequate air circulation; and building leakage.

TEMPERATURE

Temperature is a major factor in controlling the amount of BPL the air will hold. Table 1 lists the vapor pressures of BPL at various temperatures and the corresponding maximal BPL air concentration. As the temperature decreases, the maximal BPL air concentration rapidly decreases. Therefore, if BPL is used to decontaminate an enclosure at low temperatures, the amount sprayed and the spray rate should be decreased to prevent possible oversaturation. For each 10 C drop in temperature, BPL requires two or three times longer to kill the same number of organisms. Thus, for low-temperature decontamination of enclosures, less BPL should be disseminated, and a longer exposure time should be allowed. On the other hand, it is possible to decrease the exposure time by raising the ambient temperature of the enclosure. Even more important is the fact that, at higher temperatures, BPL evaporates more rapidly, and there is less chance for liquid deposition during dissemination.

RELATIVE HUMIDITY

The importance of having a relative humidity (RH) of 70% or higher when decontaminating with BPL vapor was clearly demonstrated by Hoffman and Warshowsky (6). When the humidity of the air in the structure to be decontaminated is below the 70% minimum, water must be sprayed to obtain the required level. Raising the RH is best done by injecting steam from an open autoclave or steam-line outlet, spraying water from a nebulizer, or using a vaporizer. Air should be circulated during this procedure. Just wetting the surface with water from a hose has been found unsatisfactory for raising the RH of an enclosure to the needed 70%. Care should be taken not to overhumidify the enclosure. It has been observed that water droplets hanging from a painted ceiling may absorb sufficient BPL to dissolve the paint under the droplets. Once the RH is raised in an enclosure, it is best to continue adding water vapor slowly during the decontamination period, which can be done by cracking a steam valve or by using one or more small vaporizers.

Hydrolysis of BPL vapor, even in the presence of a very high relative humidity, is not a major concern. The rate of hydrolysis of BPL in air at 100% RH is about one-half that in water. BPL at 25 C in water has a half-life of 3.5 hr, and in air, at 100% RH, the half-life is about 7 hr. At lower RH, the hydrolysis rate would be proportionately less. It is thus obvious the BPL in an enclosure cannot be eliminated in less than a day by merely raising and maintaining a high RH.

AQUEOUS BPL SOLUTIONS

An aqueous solution of BPL is often sprayed to raise the relative humidity and to disseminate the BPL in one operation. Once such a practice is started, it usually becomes routine thereafter to spray an aqueous BPL solution, regardless of the RH. At times, during the summer, the ambient RH is high, and under these circumstances the air can hold very little more moisture. If water is sprayed along with the BPL, the air becomes oversaturated and fallout results, producing a deleterious BPL solution on surfaces.

Spraying BPL with water complicates the problems of decontamination, because the droplets disseminated by a generator are a mixture of water and BPL; since the vapor pressure of water is higher, it will evaporate first. Evaporation of the water droplets will have a cooling effect, so that more time will be required for the BPL to vaporize, thus increasing the chances for fallout and collection on a surface.

Because of these factors, it is strongly recommended that aqueous BPL solutions should not be employed in enclosure decontamination. If it is necessary to raise the humidity, the water should be sprayed separately.

AIR CIRCULATION

A major factor in BPL decontamination is adequate air circulation by fans or by existing recirculating systems during dissemination of the chemical. Because the vapor pressure of BPL is so low, air in the vicinity of the spray will be rapidly saturated unless it is constantly diluted with fresh air. Ideally, the dispenser should deliver only vapor. However, if used properly, a nebulizer that disseminates small liquid droplets (less than about 30 μ) is also satisfactory. If a vapor-type dispenser is used, the air must be circulated constantly to prevent oversaturation, which leads to condensation and fallout. If the nebulizer-type dispenser is used, the air must be circulated more rapidly to prevent fallout and to permit sufficient time for evaporation. The BPL output rate of any type of dispenser must not be greater than the amount the recirculated air can hold. There must be a greater air circulation rate than spray rate, because the recirculated air will be capable of holding less and less BPL as it becomes more concentrated during dissemination and recirculation. For this reason, it is recommended that BPL be disseminated into an enclosure slowly over a longer period of time.

If the dispenser output is 100 ml of BPL per min, 500 cubic ft of air per min is required to take up the BPL vapor without oversaturation at 25 C (Table 3). The same amount of BPL would require 690 cubic ft of air per min to prevent oversaturation at 20 C. Since the air will gain more and more BPL vapor as it is recirculated during the dissemination period, the air circulation rate must be greater than 500 cubic ft/min at 25 C or 690 cubic ft/min at 20 C. As a rule of thumb, it is suggested that the amount of air circulated be twice the volume required to hold the amount sprayed. Thus, in the example cited above, at 25 C, the air circulation rate should be about 1,000 cubic ft/min. If the sprayer delivers 200 ml of BPL per min, the circulation rate should be 2,000 cubic ft/min, etc.

Table 3. *Minimal amount of air at different temperatures required for complete vaporization of 0.0264 gal (100 ml) of BPL*

Temp	Air
C	ft^3
10	1,350
15	970
20	690
25	500
30	367
35	266

In the decontamination of large areas, the air should be recirculated throughout the structure, especially during BPL dissemination. This is not always possible without a built-in recirculation system, although in some structures the air can be recirculated by a system of fans. If the structure lacks a recirculating system and has many small rooms, a number of small dispensers should be set up throughout, and fans should be employed to assure adequate distribution.

GENERATORS

Spiner and Hoffman (10) initially stated that almost any commercial insecticide sprayer could be used to disseminate BPL if the chemical did not "rain out" or thermally decompose. As stated above, a generator that delivers only BPL vapor is most desirable. It is not advisable to use ordinary thermal-type generators because of their high operating temperature. BPL decomposes to some extent even at its boiling point (162 C). Thermal-type generators operate at considerably higher temperatures than the boiling point of BPL, so some decomposition will occur; the amount depends on the temperature and the exposure time.

An even more unfavorable situation develops when an aqueous solution of BPL is sprayed by a thermal generator, because BPL hydrolyzes rapidly at high temperatures. For example, the half-life of BPL in water at 25 C is 3.5 hr, whereas, at 75 C, it is only 5 min. No data are available on the hydrolysis rate at 200 to 300 C, the lower temperature range at which a thermal generator operates. It is obvious that the hydrolysis rates will usually be extremely high under these circumstances. Therefore, aqueous BPL should never be sprayed with a thermal-type generator.

Little consideration had been given to the flash point of the chemical when a thermal-type generator was used for BPL. By the open-cup method, BPL has a flash point of 74 C. To our knowledge, no fires have resulted from spraying BPL with a thermal generator, but this may have been only fortuitous.

The nebulizer-type dispenser seems to be the most satisfactory for the purpose as long as the particle size is less than about 30 μ and the air is circulated at a sufficient rate to prevent oversaturation, condensation, and fallout.

BPL TOXICITY AND DETECTION

The carcinogenic activity of BPL has been reported by Walpole et al. (11), Roe and Glendenning (9), Palmes et al. (8), and Karlson and Weed (7). These investigators showed that frequent subcutaneous or topical applications of dilute BPL produced tumors in rats and mice. According to Karlson and Weed (7), however, BPL appears to be a weak carcinogen. Because it is a carcinogen, Spiner and Hoffman (10) recommended that repeated exposure to even subirritating doses be avoided. They also stated that the lowest concentration of BPL detectable by smell is about 0.05 mg/liter of air. To avoid reliance on human olfaction, a Chemical Corps gas-detection tube was found satisfactory for detecting trace amounts of BPL (10). Tests in this laboratory show this tube to be very sensitive, since it detects as little as 0.004 mg of the lactone. Thus, if a 1-liter air sample is taken, it is possible to detect a BPL concentration as low as 0.004 mg/liter. In a 250-ml sample, the lowest detectable concentration is 0.016 mg/liter. If the blue color does not appear (with the addition of sodium hydroxide) in the tube after taking a 1-liter sample in an aerated room after BPL decontamination, it is safe for unmasked personnel to re-enter. We calculate that 10 air changes, vented to the outside, will reduce the BPL concentration below the detectable level. However, since BPL vapor does adsorb on the surfaces, it may require a relatively long time to desorb. For this reason, an area aerated by forced ventilation may not show any detectable BPL by the tube method, but, if the aeration is discontinued and the area is closed, an irritating concentration of BPL may accumulate because of desorption from surfaces. To avoid this possibility, aeration, even reduced if necessary, should be continued for a short period after reoccupying the enclosure.

LITERATURE CITED

1. ALG, R. L., G. J. HARRIS, AND M. S. BARBEITO. 1964. Disinfection with beta-propiolactone. Soap Chem. Specialties **40**:97–100.
2. BARBEITO, M. S. 1966. Emergency disinfection of operating room and patient ward with beta-propiolactone. Hospitals **40**:100–106.
3. BRUCH, C. W. 1961. Decontamination of enclosed spaces with beta-propiolactone vapor. Am. J. Hyg. **73**:1–9.
4. DAWSON, F. W., H. J. HEARN, AND R. K. HOFFMAN. 1959. Virucidal activity of β-propiolactone vapor. I. Effect of β-propiolactone vapor on Venezuelan equine encephalomyelitis virus. Appl. Microbiol. **7**:199–201.
5. DAWSON, F. W., R. J. JANSSEN, AND R. K. HOFFMAN. 1960. Virucidal activity of β-propiolactone vapor. II. Effect on the etiological agents of smallpox, yellow fever, psittacosis, and Q fever. Appl. Microbiol. **8**:39–41.
6. HOFFMAN, R. K., AND B. WARSHOWSKY. 1958. *Beta*-propiolactone vapor as a disinfectant. Appl. Microbiol. **6**:358–362.
7. KARLSON, A. G., AND L. A. WEED. 1966. *Beta*-propiolactone as used in sterilization of homografts not carcinogenic to mice. Mayo Clinic Proc. **41**:24–28.
8. PALMES, E. D., L. ORRIS, AND N. NELSON. 1962. Skin irritation and skin tumor production by beta-propiolactone (BPL). Am. Inc. Hyg. Assoc. J. **23**:257–264.
9. ROE, F. J. C., AND D. M. GLENDENNING. 1957. The carcinogenicity of β-propiolactone for mouse skin. Brit. J. Cancer **10**:357–362.
10. SPINER, D. R., AND R. K. HOFFMAN. 1960. Method for disinfecting large enclosures with β-propiolactone vapor. Appl. Microbiol. **8**: 152–155.
11. WALPOLE, A. L., D. C. ROBERTS, F. L. ROSE, J. A. HENDRY, AND R. F. HOMER. 1954. Cytotoxic agents. IV. The carcinogenic action of some monofunctional ethyleneimine derivatives. Brit. J. Pharmacol. **9**:306–323.